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The influence of moisture on the *in vitro* embryo germination and morphogenesis of babassu (*Orbignya phalerata* Mart.)

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ABSTRACT. The objective of this study was to evaluate the physiological quality of babassu (*Orbignya phalerata* Mart.) embryos as a function of desiccation. The fruits were placed in a convection oven at 37±2°C for 0, 3, 6, 9, or 12 days. After these periods, the moisture contents of the fruits and seeds were determined. A reduction in the water content occurred more rapidly in the fruits than in the seeds. The decrease in seed moisture content from 12.59% (on the basis of fresh weight) to 4.56% did not affect the percentage of germinated zygotic embryos; however, we did observe a decline in the vigor as the seed moisture content decreased. Embryos with a 4.56% moisture content developed into seedlings of shorter lengths after 30, 60, and 90 days of *in vitro* growth. The longest seedlings originated from embryos with a 10.42% moisture content after 90 days. Thus, it was evident that the babassu embryos were tolerant to desiccation, whereas the seedling length was negatively affected by a reduction in the seed moisture content. Therefore, babassu fruits may be dehydrated and stored without losing their viability, which thereby guarantees the conservation of their germplasm.

Keywords: Arecaceae, desiccation tolerance, tissue culture.

Influência da umidade na germinação e morfogênese *in vitro* de embriões de babaçu (*Orbignya phalerata* Mart.)

RESUMO. O objetivo deste estudo foi avaliar a qualidade fisiológica de embriões de babaçu (*Orbignya phalerata* Mart.) em função da dessecação. Os frutos foram mantidos em estufa de circulação forçada a 37±2°C por 0, 3, 6, 9 e 12 dias. Após esses períodos, foi determinado o teor de água dos frutos e sementes, sendo também avaliado a percentagem de embriões zigóticos germinados *in vitro*, GRI (definir) e desenvolvimento das plântulas. A redução no teor de água ocorre mais rapidamente em frutos que em sementes. A diminuição no conteúdo de água da semente a partir de 12,59% (umidade inicial) para 4,56% não afeta a porcentagem de germinação *in vitro* dos embriões zigóticos, no entanto foi observado diminuição no vigor dos embriões à medida que decresce o teor de água da semente. Embriões com 4,56% de umidade desenvolveram as menores plântulas após 30, 60 e 90 dias de cultivo *in vitro*. As maiores plântulas foram obtidas de embriões com 10,42% de umidade, após 90 dias. Diante disso, fica evidente que os embriões de babaçu toleram a dessecação, mas que a altura das plântulas foi afetada negativamente pela redução do teor de água das sementes.

Palavras-chave: Arecaceae, tolerância à dessecação, cultura de tecidos.

Introduction

Among Brazilian species of the Arecaceae family (palm), babassu (*Orbignya phalerata* Mart.) trees feature a simple, robust trunk that can grow up to 20 m tall. The fruit grows in bunches, and each tree can produce up to 2,000 fruit annually (Machado et al., 2006). This species is solitarily distributed in forests or in open areas, and is most commonly found in degraded areas where the species is a dominant pioneer. The babassu palm is native to the central western, northern, and northeastern regions of Brazil. It inhabits extensive massif forests located in the transition between the humid forests of the Amazon basin, savannas, and semi-arid lands of northeast Brazil, covering approximately 18 milions hectares distributed in the states of Maranhão, Piauí, Tocantins, Goiás, Amazonas, Pará, and Mato Grosso (LORENZI et al., 1996; SILVA et al., 2001).

The babassu is a palm tree used in landscaping and the production of fibers, perfume, soap, lubricants, cooking oil, and medicinals. Babassu is also regarded for its application in the fuel industry as a form of biodiesel. Due to efficient processing methodologies, the oil from the seeds offers a 67% extraction yield and can be used in a pure form or mixed with diesel gasoline in diesel cycle engines without any necessary adaptations of the machines (LIMA et al., 2007; LORENZI et al., 1996).

The use of biomass for biofuel production has inspired significant economic interest in palm trees, mainly macaúba (*Acrocomia aculeata* Jacq. Loddiges ex Mart.), oil palm (*Elaeis guinnensis* Jacq.), inajá (*Maximiliana regia* Mart.), tucumã (*Astrocaryum aculeatum* Meyer), and babassu (*Orbignya phalerata* Mart.) (RAMOS et al., 2003; MARTINS et al., 2009). However, the germination of species in the Arecaceae family is slow, irregular, and often has a low success rate. These aspects of the germination of the Arecaceae species have been attributed to the following factors: the maturity of the seed at harvest, the presence or lack of a pericarp, the time between harvesting and sowing, physical dormancy, the environmental temperature, the substrate, the water content, and storage. It is important to consider these factors in conservation practices, as they can influence the germination, quality, and health of future plants (MARCOS FILHO, 2005).

The effects of desiccation on the seed germination rate and the storage tolerance are used for the classification of seeds into physiological groups, in which the majority of palms show a 'recalcitrant' behavior. These palms include palmito (*Euterpe edulis* Mart.), açaí (*Euterpe oleraceae* Mart.), areca palm (*Chrysalidocarpus lutescens* Wendl.), and peach palm (*Bactris gasipaes* Kunth) (BECWAR et al., 1982; FERREIRA; SANTOS, 1992; ARAÚJO et al., 1994; BOVI et al., 2004; MARTINS et al., 2006).

Given the difficulties of germinating this species, *in vitro* embryonic cultivation is a promising technique to advance the knowledge of species that are difficult to propagate by other means, thus allowing for the study of their embryonic development, dormancy disruption, and seedling production. Moreover, embryonic palm tree cultures, which usually undergo slow germination, can be very useful for reducing the time required for the production of the plant. Embryonic tissue explants are also very useful in studies of *in vitro* clonal propagation because of their juvenile nature and high regenerative potential (PEREIRA et al., 2006). Although this process involves several stages that follow a defined protocol, clonal propagation can be optimized to obtain high-quality seedlings with low production and time costs (PEREIRA et al., 2007).

Because there have been few studies of this species of Arecaceae to date, there is a lack of knowledge on the germination processes and the techniques that will enable the conservation of the babassu palm. Therefore, this study aimed to evaluate the physiological quality of babassu embryos as a function of the desiccation of the seeds.

Material and methods

The study was conducted at the Seed and Plant Tissue Culture Laboratories of the Federal Institute of Goiás, Rio Verde Campus, Goiás State. Babassu fruit (*Orbignya phalerata* Mart.) was collected in October 2009 from plants of unknown ages under natural cerrado conditions from the Gameleira Farm in the municipality of Montes Claros de Goiás, Goiás State (16° 07'S - 51° 18'W, altitude of 592 m) (Figure 1A).

Desiccation

After the extraction of the bract via pruning with pliers, the selected fruit were classified into 4 groups to homogenize the replicates: small (100.00-150.00 g), medium (150.01-200.00 g), large (200.01-250.00 g), and extra-large (250.01-300.00 g). These fruits were used equally in all of the replications. The mean weights, lengths, and diameters are shown in Table 1.

Table 1. Classification of babassu fruit according to the mean weight, length, and diameter.

Unit	Small	Medium	Large	Extra-large
Weight (g)	128.7 (±19.2)*	174.8 (±14.6)	220.2 (±13.5)	271.3 (±14.4)
Length (cm)	93.4 (±6.4)	97.7 (±8.6)	103.9 (±9.2)	108.3 (±7.6)
Diameter (cm)	56.2 (±4.3)	61.9 (±3.5)	64.2 (±3.7)	68.6 (±6.0)

*(±Standard deviation of the mean).

Whole fruits (exocarp, mesocarp, and endocarp) were covered with a 2-cm diameter mesh galvanized screen and placed in a Marconi oven (Model MA 035). The fruits were dried with continuous air renewal and circulation at 37±2°C for 0, 3, 6, 9, or 12 days to obtain their respective water contents of both the fruits and seeds (endocarp or nut) after each predetermined time period (Figure 1B). The water contents were determined after drying in a 105±2°C oven until a constant mass relative to the fresh weight was obtained; 4 replicates of 10 fruit and seeds were used. A second batch of fruit was removed from the oven and broken with a mechanical press to obtain 30 embryos with the goal of evaluating the effect of dehydration on *in vitro* germination (Figure 1B).

The experimental design was completely randomized. For the humidity variable, we performed a variance analysis with the F test and compared treatment means with the Tukey test (5%). The other variables were analyzed by polynomial regression.

In Vitro germination

Zygotic embryos were removed with a scalpel and placed in acrylic Gerbox® boxes with distilled water until all of the embryos were removed (Figure 1E). The embryos were then covered in gauze, sterilized for 30 seconds in 70% alcohol and 20 minutes in a 20% solution of sodium hypochlorite (NaOCl; commercial bleach - 2.5% active chlorine), and washed three times with sterile water (Figure 1E).

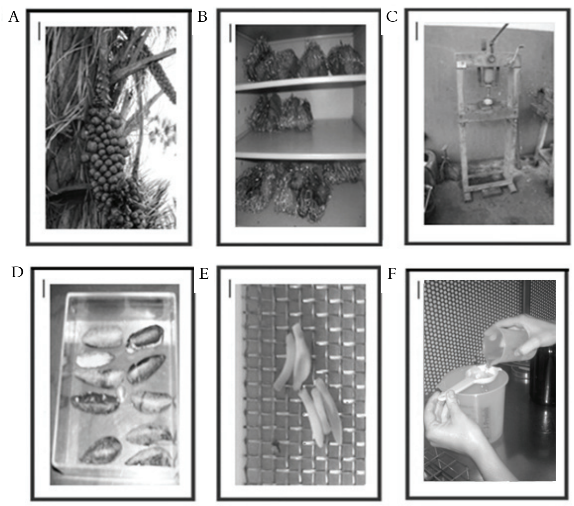

Figure 1. Methodology for the collection and dehydration of fruits and the extraction and sterilization of zygotic embryos of babassu (*Orbignya phalerata* Mart.). A) Sampling. Bar = 0.5 m. B) Drying in a convection oven at 37°C for up to 15 days. Bar = 30 cm. C) Breaking of the fruit in a hydraulic press. Bar = 30 cm. D) Seeds extracted with knives and box cutters. Bar = 2 cm. E) Embryos extracted with a scalpel and tweezers. Bar = 3 mm. F) Disinfection of the embryos covered in gauze. Bar = 2 cm.

The embryos were inoculated in test tubes containing 20 mL of MS medium (MURASHIGE; SKOOG, 1962) with 50% salts supplemented with 30 g L^{-1} sucrose and 0.1 g L^{-1} myo-inositol in 3.5 g L^{-1} solidified agar. The pH of the medium was adjusted to 5.7±0.1 before autoclaving at 121°C and a pressure of 1.05 kg cm^{-2}.

After inoculation, the embryos were kept in a growth chamber at 25±3°C under a 16-hour photoperiod with a photosynthetically active radiation of 40-60 μmol m^{-2} s^{-1} provided by fluorescent lamps. The culture medium (as described above) was changed monthly under laminar flow conditions.

Germination was counted daily to calculate the GRI (germination rate index), according to the formula proposed by Maguire (1962), and the germination percentage. The seedling explant length was assessed after 30, 60, and 90 days.

The experimental design was completely randomized, and 30 replicates were used for each moisture content test. A pooled analysis of data relative to the GRI, germination percentage, and seedling length was performed via polynomial regression.

Results and discussion

Drying curve

We observed a decrease in the water content of the fruit early in the drying process, with a reduction of 21.89 to 16.70% and 21.89 to 13.77% moisture after 6 and 12 days of drying, respectively, representing a total loss of 37.09% of the initial moisture content at the end of drying after 12 days (Figure 2A). The initial seed moisture content was much lower than that of the fruit, and the dehydration process in seeds occurred more slowly during the drying period. After 12 days, the seed moisture content totaled 4.56%, which represents a 63.78% decrease relative to the initial water content (12.59%) (Figure 2B).

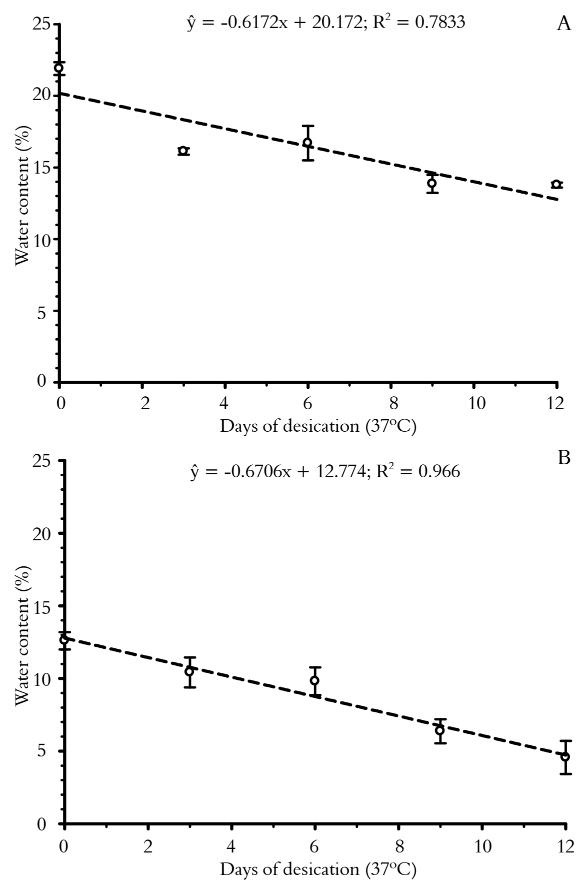

Figure 2. Water loss in the fruit (A) and seeds (B) of babassu (*Orbignya phalerata* Mart.) subjected to different drying periods in a convection oven at 37°C.

Upon regression analysis, it was concluded that the high coefficients of determination (78.33 and 96.60% for the fruits and seeds, respectively) suggested that the model was a good fit to the water content data, and the drying time was closely related to the water content. The fruits and seeds concurrently lost water in the same proportion.

When the coefficient values were compared, it was possible to establish a statistical relationship between the two studied plant materials (fruits and seeds) to obtain a linear fit where $R^2 = 0.7197$ (Figure 3). Due to its fibrous pericarp and mealy flesh, the free water in the deeper tissues of the fruit was lost at the same rate as the water in the more superficial tissues closer to the exocarp. Although the water loss was similar between the fruits and seeds, the initial water contents were different. Therefore, even though water was lost at the same proportion, the total water loss was much higher for the seeds than the fruits because the initial water content in the seeds (12.59%) was lower than that of the fruits (21.89%).

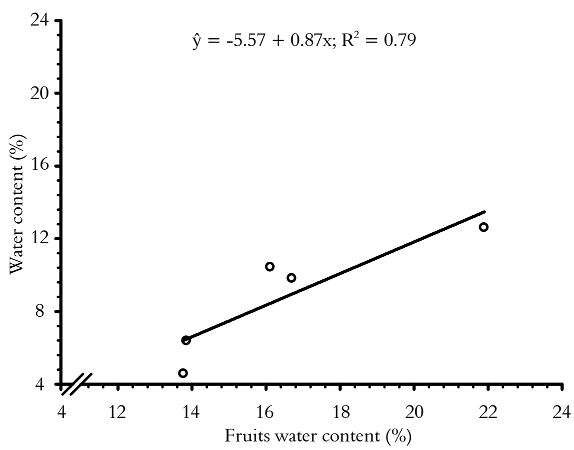

Figure 3. The relationship between the water content in the fruits and seeds of babassu (*Orbignya phalerata* Mart.) subjected to different drying periods in a convection oven at 37°C.

In vitro germination

The reduction in the water content of the seeds from 12.59 to 4.56% did not affect the in vitro germination rate in the zygotic embryos of babassu, as the germination percentage ranged between 93.3 and 86.7% (Figure 4).

However, vigor, which is represented by GRI, was negatively influenced by a reduction in the embryo moisture content with an approximately 50% loss when the embryo reaches 4.56% of its water level and shifts from 0.14 (initial) to 0.071. It is possible that drying caused physiological changes in the cellular membranes of the embryo, thus reducing the vigor. When cells are hydrated, their membranes maintain a lipid bilayer in a liquid crystalline state at biological temperatures. With desiccation, the phospholipid arrangement in the membranes changes from a lamellar configuration to a hexagonal configuration, causing membrane dysfunction (FARRANT et al., 1988).

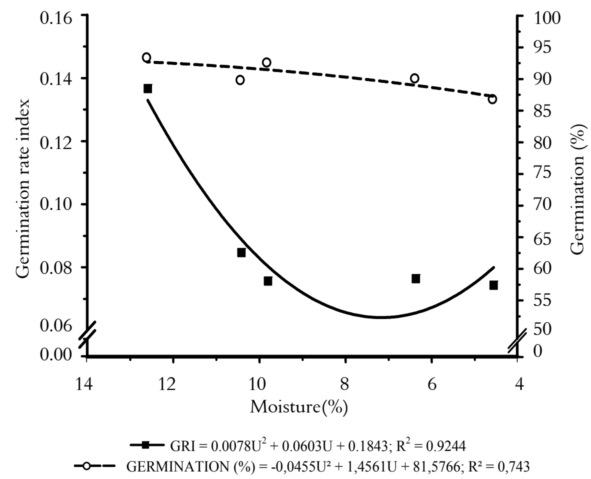

Figure 4. The germination percentage and germination rate index (GRI) of babassu (*Orbignya phalerata* Mart.) embryos subjected to different drying times in a convection oven at 37ºC.

The measurements of the seedling lengths after 30, 60, and 90 days of *in vitro* cultivation revealed that the embryos with a 4.56% moisture content upon inoculation resulted in shorter seedlings, reaching 1.25, 2.05, and 2.73 cm, respectively. The embryos with other initial moisture contents were evaluated at the same intervals; it was found that their lengths were uniform, especially those seedlings arising from embryos with a 12.6% moisture content, which produced seedlings of 1.65 cm at 30 days in culture. Embryos with a moisture content of 10.42% produced seedlings of 2.72 and 4.68 cm in length, at 60 and 90 days of culture, respectively, demonstrating the largest lengths observed for each season (Figure 5).

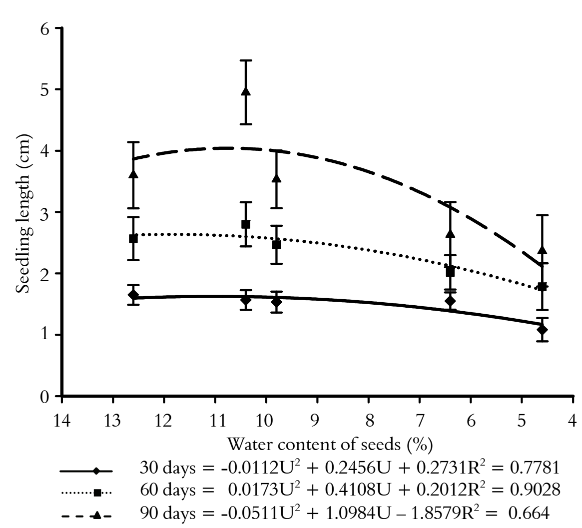

Figure 5. Seedling length (cm) of babassu (*Orbignya phalerata* Mart.) embryos grown *in vitro* for 30, 60, and 90 days with different initial moisture contents.

In the evaluation performed after 30 days, the seedlings that were derived from embryos with different water contents achieved an average of 1.48 cm of growth; this value was very similar among the different water content trials. At 60 days, there was a greater difference in seedling growth, especially between embryos with moisture contents of 4.56 and 10.42% (with a difference of 2 cm), making it clear that the loss of greater than 50% of the initial moisture content of the seed interfered with the seedling length over time. This difference became more pronounced after 90 days of *in vitro* growth, where embryos with moisture contents of 10.42 and 12.59% reached seedling lengths of 4.5 and 3.5 cm, respectively (Figure 6).

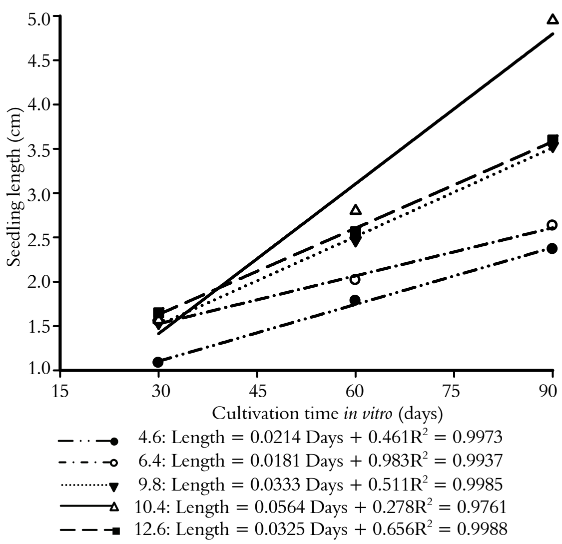

Figure 6. Seedling length (cm) of babassu (*Orbignya phalerata* Mart.) grown *in vitro* for 30, 60, and 90 days from zygotic embryos with different moisture contents.

It is noteworthy that the process that makes seed storage possible (i.e., the promotion of a low moisture content through drying) can also be harmful to the embryo. Therefore, it is of great importance that this process be performed in ovens that are temperature controlled to maintain the germination potential and vigor of the seeds (CHIN, 1988).

Conclusion

The seeds of this species showed an orthodox behavior, tolerating desiccation to low water contents, which can further facilitate their storage and extend the conservation of the babassu palm with the goal of replacing natural populations.

The reduction of the seed water content from the initial conditions, independent of the drying time, did not reduce the seed germination percentage but reduced the vigor by approximately 50%.

Seed germination occurred at low water contents, indicating an orthodox seed behavior; further studies will be required to demonstrate this seed characteristic.

The seedling length was negatively affected by a reduction in the seed moisture content.

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